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Influence of seed priming agents on yield, yield parameters and purple seed stain disease in soybean*

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Abstract: Seeds of JS 335 variety were primed with different fungicides and bio-agents and were subjected for seed germination and field performance in order to know the influence of fungicides and biocontrol agents on germination, seedling growth, disease incidence and yield parameters. Under laboratory condition, combi-fungicides carboxin 37.5 + thiram 37.5 WS @ 0.2% recorded significantly higher seed germination and root length. Whereas, *Trichoderma harzianum* @ 0.6% recorded maximum shoot length and significantly higher seedling length. Seed priming with tebuconazole 5 EC @ 0.2%, captan 70 + hexaconazole 5 WP @ 0.2%, and carbendazim 25 + mancozeb 50 WS @ 0.2% recorded significantly higher seedling dry weight as compared to control. The field experiment indicated that the *Bacillus subtilis* @ 0.6% recorded significantly higher seed yield followed captan 70 + hexaconazole 5 WP @ 0.2% and carbendazim 25 + mancozeb 50 WS @ 0.2%. The increase in seed yield was attributed to increased number of pods per plant, pod weight, seed weight in primed treatments as compared to control. The incidence of purple seed stain (PSS) disease was less in these treatments as compared to control.

Key words: Bio-inoculant, Purple seed stain disease, Seed priming, Soybean

Introduction

Soybean (*Glycine max* (L.) Merrill.) belonging to family papilionaceae, possess a very high nutritional value. It contains about 20 per cent oil and 40 per cent high quality protein, 30 per cent carbohydrates, 4 per cent saponins and 5 per cent fiber. The oil contains about 0.5-1.0 per cent lecithin which is essential for building up of human nerve tissues. Due to high protein content, soybean is known as 'poor man's meat'. Soybean protein is rich in valuable amino acid lysine (5%) which in most of the cereals is deficient. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of Vitamin A and C. In India, soybean production and its quality has to be increased to compete in the world for export. The quality of soybean produce is affected by the biotic factors, predominantly by purple seed stain disease.

Seed treatment with microbial antagonists or fungicides protects the seed from infection by seed and soil-borne pathogens, enables the seed to germinate and establish as a healthy seedling (Windels, 1981). Seed treatment is therefore a routine practice to ensure good emergence and better crop stand (Ramos and Ribeiro, 1993). There are diverse opinion whether legume seeds should be treated with fungicides and whether seed dressing materials might adversely affect the *Rhizobium* spp. and hence the nodulation.

A successful antagonist should colonize rhizosphere during seed germination (Weller, 1983). Seed priming with PGPRs increase germination and improve seedling establishment. It initiates the physiological process of germination, but prevents the emergence of plumule and radicle. Initiation of physiological process helps in the establishment and proliferation of PGPRs on the spermosphere (Taylor and Harman, 1990). Bio-priming of seeds with bacterial antagonists increase the population load of antagonist to a tune of 10 fold on the seeds, thus protect the rhizosphere from plant pathogens (Callan *et al.,* 1990).

Purple seed stain disease of soybean is caused by the fungus *Cercospora kikuchii* which is considered to be the fifth most important yield-depressing pathogen in the world (Sinclair and Hartman, 1999). The major problem with this disease is reduction in the visual quality of the seed, making it unsuitable for many sectors of the industry. Purple seed stain was first reported in Korea (Suzuki, 1921). As the name suggests affected seeds are discoloured, with pink to dark purple areas occurring as small blotches or covering the entire seed coat. Symptoms also occur on leaves (purplish discolouration of the upper leaf surfaces and lesions on the petioles) and stems (elongated lesions) and in severe infections premature leaf death can occur (Schuh, 1999). Symptoms are not usually visible until mid pod fills, by which time infection has already occurred. Infection can occur over a wide range of conditions, but favoured by increasing periods of dew in later season, with spores originating from infected residues.

Material and methods

Soybean variety JS 335 was taken for the study as it is susceptible for PSS disease. Pre soaked (4 hours) seeds were treated with *Rhizobium* culture @ 10 g per kg seeds using natural gum. Seed priming was done by treating the seeds with different fungicides separately and bioinoculants as per the treatments (Table 1). They were shade dried overnight by spreading on ground and then subjected for laboratory seed germination and field sowing.

Laboratory seed germination test was conducted in three replications using 50 seeds for each treatment by adopting paper towel method as described by ISTA procedures

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Table 1. Seed germination and seedling growth parameters as influenced by seed primers and purple seed stain disease score in harvested soybean seeds as influenced by seed primers

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Dry weight (mg)	PSS % incidence	PSS-PDI (%)
Tebuconazole 5 EC @ 0.2%	93.00	20.43	5.97	26.40	130	1.00	0.34
Captan 70 + Hexaconazole 5 WP @ 0.2%	90.00	15.20	2.14	17.34	130	0.00	0.00
Carboxin 37.5 + Thiram 37.5 WS @ 0.2%	99.00	19.87	9.94	29.80	120	0.00	0.00
Captan 70 WP @ 0.2%	91.00	18.14	11.09	29.23	120	0.57	0.37
Carbendazim 25 WP @ 0.2%	94.00	18.18	13.10	31.28	120	0. 33	0.11
Carbendazim 25 + Mancozeb 50 WS @ 0.2%	91.00	16.40	11.53	27.93	130	0. 23	0.11
Trichoderma harzianum @ 0.6%	93.00	16.73	14.85	31.58	120	0.33	0.11
Pseudomonas fluorescens @ 0.6%	89.00	18.78	12.58	31.35	120	0. 33	0.19
Bacillus subtilis @ 0.6%	90.00	15.28	13.80	29.08	120	0. 22	0.11
Control	89.00	17.35	11.65	29.00	110	2.33	1.30
S.Em.±	1.29	0.82	0.59	1.52	3.32	0.33	0.03
C.D. at 5%	4.12	2.63	1.88	3.85	10.00	0.97	0.10

PSS- Purple seed stain, PDI- Per cent disease index

(Anon., 1999). Seeds were incubated at slanting position in walk-in germinator room in growth cabinets. The temperature of 25±1ºC and RH of 95 per cent was maintained during the germination test. Untreated seeds served as control. After eight days the germination per cent, root length, shoot length and seedling dry weight were recorded for each treatment.

The field experiment was laid out in randomized block design with three replications and primed seeds were sown with a spacing of 30 x 10 cm as per the treatments. Each treatment was sown in plot size of 3 x 4 m. The crop was raised successfully following all other recommended package of practices. Upon crop maturity the crop was harvested treatment wise separately, and yield and yield attributes were recorded.

The PSS disease incidence was recorded in harvested grains by physical counting of infected seeds in each sample and disease scoring was done using 0-9 scale (0 for no symptoms on seed, 1 for up to 1% of seed covered by lesions, 3 for 1-10% of seed covered by lesions, 5 for 11-25% of seed covered by lesions, 7 for 26-50% of seed covered by lesions and 9 for more than 50% of seed covered by lesions). All the data recorded were subjected to statistical analysis.

The per cent disease index was calculated by using formula given by Wheeler (1969).

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{No. of seeds assessed}} \text{x} \frac{100}{\text{Maximum disease grade}}$$

Per cent disease incidence was calculated by using the formula given below.

$$\text{Per cent disease incidence} = \frac{\text{No. of infected seed}}{\text{Total no. of seeds assessed}} \text{x100}$$

Results and discussion

Increased seed germination percentage was recorded in primed seeds. Significantly maximum seed germination of 99 per cent was observed in carboxin 37.5 + thiram 37.5 WS @ 0.2% followed by 94.0 per cent in carbendazim 25 WP @ 0.2%, 93.0 per cent in both *Trichoderma harzianum* @ 0.6% and tebuconazole 5 EC @ 0.2%. The root length was significantly influenced by the seed priming. Significantly higher root length was recorded with tebuconazole 5 EC @ 0.2% priming (20.43 cm) and was on par with carboxin 37.5 + thiram 37.5 WS @ 0.2% (19.87 cm), captan 70 WP @ 0.2% (18.14 cm), carbendazim 25 WP @ 0.2% (18.18 cm) and *Pseudomonas fluorescens* @ 0.6% (18.78 cm). Significantly highest shoot length was recorded with *T. harzianum* @ 0.6% (14.85 cm) and was on par with carbendazim 25 WP @ 0.2% (13.10 cm) and *Bacillus subtilis* @ 0.6% priming (13.80 cm). The lowest shoot length was recorded with captan 70 + hexaconazole 5 WP @ 0.2% priming (2.14 cm). The seedling length was significantly influenced by the seed priming. Captan 70 + hexaconazole 5 WP @ 0.2% recorded lowest seedling length (17.34 cm), while *T. harzianum* @ 0.6% (31.58 cm) recorded significantly highest seedling length and was on par with rest of the priming treatments. Lowest dry weight (110 mg) was recorded by control. Higher seedling dry weight (130 mg) was recorded with carbendazim 25 + mancozeb 50 WS @ 0.2%, captan 70 + hexaconazole 5 WP @ 0.2% (130 mg) and tebuconazole 5 EC @ 0.2% (130 mg) and were on par with rest of the priming treatments (Table 1). The variation in seed germination percentage and seedling length may be attributed to plant growth promotional effect of seed primers especially bioagents that may produce growth regulatory substances (hormones) upon seed imbibitions as reported by Jin and Tylkowska (2005), Bapurayagouda (2010) and Park *et al.* (2010). The decline in germination percentage in untreated control may be attributed to ageing effect leading to depletion of food reserves and decline in synthetic activity of embryo apart from death of seed because of fungal invasion, insect damage, fluctuating temperature and relative humidity. Similar results were reported by Hooda and Singh (1993) in wheat.

In general there was no severity of PSS disease either in field crop or in the produce. However, the results from the investigation revealed that the per cent disease index was maximum in control (1.30%) and zero in captan 70 + hexaconazole 5 WP @ 0.2% and carboxin 37.5 + thiram 37.5 WS @ 0.2%. Similarly, control recorded highest per cent disease incidence (2.33%) as compared to other primed seeds, while lowest (0.00) was recorded in captan 70 + hexaconazole 5 WP @ 0.2% and

Table 2. Influence of seed primers on yield and yield parameters in soybean

Treatments	Days to 50% flowering	No. of pods/ plant	Pod weight/ plant (g)	Seed weight/ plant (g)	100 seed weight (g)	% increase over control	Seed yield (kg/ha)	% increase over control	Harvest index (%)
Tebuconazole 5 EC @ 0.2%	44.00	53.33	24.53	18.40	12.22	9.89	2750	1.75	0.56
Captan 70 + Hexaconazole 5 WP @ 0.2%	49.67	76.67	35.20	25.93	13.31	19.69	3116	15.29	0.61
Carboxin 37.5 + Thiram 37.5 WS @ 0.2%	41.33	52.40	20.00	18.40	11.96	7.55	2870	6.17	0.54
Captan 70 WP @ 0.2%	41.00	57.40	23.73	16.73	12.43	11.78	2863	5.92	0.53
Carbendazim 25 WP @ 0.2%	41.00	49.13	20.87	13.20	11.68	5.04	2746	1.60	0.51
Carbendazim 25 + Mancozeb 50 WS @ 0.2%	41.00	59.60	32.52	24.80	12.35	11.06	3083	14.06	0.62
Trichoderma harzianum @ 0.6%	39.33	52.53	20.93	15.67	11.58	4.14	2780	2.84	0.55
Pseudomonas fluorescens @ 0.6%	41.33	60.67	22.93	19.00	12.29	10.52	2935	8.57	0.59
Bacillus subtilis @ 0.6%	39.00	70.27	36.50	31.20	12.45	11.96	3221	19.17	0.66
Control	39.00	58.27	23.27	21.20	11.12	-	2703	-	0.47
S.Em.±	1.22	4.18	1.75	1.39	0.31	-	158.26	-	0.04
C.D. at 5%	3.63	12.43	5.19	4.13	0.92	-	470.21	-	0.11

carboxin 37.5 + thiram 37.5 WS @ 0.2% and was on par with rest of the priming treatments (Table 1).

Seed yield differed significantly with priming treatments over control (Table 2). The yield was significantly higher in *B. subtilis* @ 0.6% (31.20 g/plant) followed by captan 70 + hexaconazole 5 WP @ 0.2% (25.93 g/plant) and carbendazim 25 + mancozeb 50 WS @ 0.2% (24.80 g/plant) as compared to control (21.20 g/plant) and other treatments. It is indicated that seed priming has differential influence on the allocation of assimilates between vegetative and reproductive organs possibly by PGPR effect. In general, crop yield depends on the accumulation of photo-assimilates during the growing period and the way they are partitioned between desired storage organs of the plant. In the present study, it was revealed that the seed priming with *B. subtilis* @ 0.6%, captan 70 + hexaconazole 5 WP @ 0.2% and carbendazim 25 + mancozeb 50 WS @ 0.2% resulted in significantly increased the number of seeds, number of pods, 100 seed weight which have contributed for higher seed yield as compared to control and other treatments (Table 2). Sushma *et al.* (2003), Pederson and Lauer (2004) and Gawade *et al.* (2009) also reported similar effect in different crops.

Chaudhary (1981) opined that harvest index is the only physiological parameter which is enough to account for variation in yield potential in soybean. It differed significantly due to priming in the present study and high yielding treatments *viz., B. subtilis* @ 0.6%, *P. fluorescens* @ 0.6% and captan 70 + hexaconazole 5 WP @ 0.2%, carbendazim 25 + mancozeb 50 WS @ 0.2% showed higher harvest index indicating that these treatments enhanced translocation efficiency of assimilates to economic parts.

Seed priming with fungicides and biocontrol agents plays vital role in increasing germination percentage and results in proper plant stand and healthy seedlings. Seed priming with *B. subtilis* @ 0.6%, captan 70 + hexaconazole 5 WP @ 0.2%, and carbendazim 25 + mancozeb 50 WS @ 0.2% were found effective in increasing yield by 10-15 per cent besides controlling the PSS disease as compared to control.

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